Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095)

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This paper results from the final phase of the ENDO project (DGXII AIRII-CT94-1095), a European Commission-funded project on non-digestible oligosaccharides (NDO). All participants in the programme met to perform a consensus exercise on the possible functional food properties of NDO. Topics studied during the project (including a workshop on probiotics and prebiotics) and related aspects, for which considerable evidence has been generated recently, were evaluated on the basis of existing published scientific evidence. There was a general consensus that: (1) there is strong evidence for a prebiotic effect of NDO in human subjects. A prebiotic effect was defined as a food-induced increase in numbers and/or activity predominantly of bifidobacteria and lactic acid bacteria in the human large intestine; (2) there is strong evidence for the impact that NDO have on bowel habit; (3) there is promising evidence that consumption of inulin-type fructans may result in increased Ca absorption in man; (4) there are preliminary indications that inulin-type fructans interact with the functioning of lipid metabolism; (5) there is preliminary evidence in experimental animals of a preventive effect against colon cancer. Human nutrition studies are needed to substantiate these findings. It was concluded that the nutritional properties of NDO may prove to be a key issue in nutritional research in the future.

Functional foods: Non-digestible oligosaccharides: Prebiotics

Dietary carbohydrates represent a complex group of food components which, besides their usefulness for food technologists, have a variety of physiological and nutritional properties in man. According to the scientists who participated in the discussion, it has become clear over the last decade that the group of non-digestible oligosaccharides (NDO) play, or are likely to play, an important nutritional role (Cummings *et al.* 1997). As is shown in Table 1, NDO are composed of a variety of monosaccharides that are linked in several ways (type of bond and chain length) and typically are composed of a polydisperse set of comparable molecules differing only in chain length. The available NDO preparations are either (1) extracted from natural sources (e.g. inulin, soyabean oligosaccharides) followed by partial enzymic hydrolysis (e.g. xylo-oligosaccharides, oligofructose, malto-oligosaccharides), or (2) synthesized by subjecting disaccharides such as lactose or sucrose to the action of transferases (e.g. transgalacto-oligosaccharides).

NDO such as the fructans and oligosaccharides from beans have always been present naturally in human plant foods, although total daily intakes are largely unknown. In populations consuming a Western-style diet, the intake of inulin-type fructans has been estimated to range between 1 and 4 g/d (Van Loo *et al.* 1995).

Although differing in their chemical characteristics, all the NDO resist digestion in the human small intestine and they are potential substrates for the bacteria that colonize the large intestine.

Here, we report the consensus reached by the authors, who collaborated for a period of more than 3 years in a shared-cost research programme funded by the European Commission (DGXII AIRII-CT94-1095) and known as the ENDO project (European project on non-digestible oligosaccharides). In reaching this consensus, the authors considered both the results of their own research in the ENDO project and the data available in the scientific literature. One

Table 1. Non-digestible oligosaccharides (NDO) that were studied in the ENDO project*

Name	Type of bond and composition/ Production method	DP	Digestibility†	Allowed for human consumption in:	RSCC‡ (g/kg)
Xylo-oligosaccharides (350 or 700 g OS/kg)	$\beta\,(1\rightarrow4)$-Linked xylose moieties/ Enzymic hydrolysis of xylan	2–4	– – –	Japan	294
Soyabean oligosaccharides (350 g OS/kg)	Raffinose (F-Gal-G) and stachyose (F-Gal-Gal-G) mixture/ Extraction from soyabean whey	3–4	–	Japan, USA	322
Transgalactosylated oligosaccharides (550 or 850 g OS/kg)	6′ Galactosyllactose (with *Aspergillus oryzae* β-galactosidase); $\beta\,(1\rightarrow4)$ 4′ Galactosyllactose (with *Cryptococcus laurentii* β-galactosidase); $\beta\,(1\rightarrow6)$/ Transgalactosylation of lactose	2–8, mostly 3	– – –	Japan, some EC countries	NM
Palatinose condensates (950 g OS/kg)	Enzymically rearranged sucrose molecules (Palatinit) subsequently pyrolysed. Various types of bonds	2–7	– – to –	Japan	463
Isomalto-oligosaccharides (500 or 900 g OS/kg)	$\alpha(1\rightarrow4)$ G, branched via $\alpha(1\rightarrow6)$ G/ Transgalactosylation of maltose	2–8	– to +	Japan	211 298
Inulin	$\beta(2\rightarrow1)$ Fructans/ Chicory root extraction	2–65	– – –	EC, USA, Japan	858
Oligofructose	$\beta(2\rightarrow1)$ Fructans/ Hydrolysis of chicory inulin or transfructosylation from sucrose	2–8 3–5	– – –	EC, USA, Japan	874
Pyrodextrin	Complex mixture/ Pyrolysis of maize or potato starch	Complex mixture	– –	Japan	200

F, Fructose; G, Glucose; Gal, Galactose; DP, degree of polymerization; RSCC, resistant short-chain carbohydrates; OS, oligosaccharides; EC, European Community; NM, not measured.
* The criteria taken into consideration for human feeding studies were the commercial availability and the approval for human consumption in at least one country. Some promising NDO are also shown for comparative *in vitro* research.
† (– – –), >90 % arrives in the colon; (– –), between 50 and 90 % arrives in the colon; (–), about 50 % arrives in the colon; (+), digestible.
‡ As analysed by the method of M Quigley and H Englyst (unpublished) developed as part of the ENDO project. Values are means of three analyses.

of the objectives of the ENDO project was to contribute toward answering the question: are NDO functional food ingredients? Although not yet completely agreed on by the scientific community, the concept of functional foods refers to: 'foods similar in appearance to conventional foods that are consumed as part of a normal diet and have demonstrated physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions' (Clydesdale, 1997).

This consensus report aims to evaluate current scientific knowledge on the nutritional properties of NDO in light of the emerging concept of functional food science. The evidence was categorized as strong (based on confirmed human feeding studies), promising (human feeding studies needing confirmation) or preliminary (experimental animal studies). In addition, this consensus report makes proposals for future research.

Consensus report

This consensus report is divided into five sections: (1) analytical aspects; (2) bowel habit and gut flora; (3) mineral absorption; (4) lipid metabolism; and (5) experimental effects in relation to colon cancer. In each section, the state of the art is summarized, with emphasis on the relevant publications, followed by a report on the new developments generated during the ENDO project and, finally, the consensus reached by the group on the particular issue.

During the preparation of this report (early 1998) more work was published on NDO. These data are included in the state-of-the-art sections.

Definition, classification and analysis

State of the art. In the International Union of Pure and Applied Chemistry - International Union of Biochemistry (IUPAC-IUB) nomenclature, an oligosaccharide is defined as being composed of two to about ten monomers linked together, but some of the NDO under discussion have a chain length greater than this. Although the NDO are very similar to true oligosaccharides from a physiological point of view, physiological variables cannot be used as the only criteria for classification. A practical working approach to distinguish NDO from other groups of carbohydrates is proposed. Analytical methods have been developed to identify, characterize and quantify NDO as pure chemicals, as mixtures and as components of complex food products. Methods are available for quantification of the inulin-type fructans (Quemener *et al*. 1994; Van Loo *et al*. 1995; Hoebregs, 1997).

ENDO contribution. It has been demonstrated that the NDO can be distinguished analytically from poly-saccharides on the basis of their solubility in ethanol (800 ml/l), at pH 2, at a temperature of 0° for 30 min. These conditions are pragmatic and easily reproducible. NDO (termed resistant short-chain carbohydrates by Englyst's

group) remain in solution after the dispersal and hydrolysis of starch and the precipitation of NSP in the Englyst procedure for determination of NSP (Englyst *et al.* 1994).

As part of the present study, a GLC procedure was developed for the measurement of total NDO as the sum of their constituent sugars, which includes the option of specific determination of fructans. In the procedure, mono- and disaccharides are measured directly and subtracted from the total carbohydrates in solution to give values for the NDO. In some studies, particularly fermentation measurements, it is important to be able to quantify individual NDO. High-performance anion exchange chromatography with pulsed amperometric detection was chosen for this purpose, as this technique allows separation of carbohydrates with a degree of polymerization from 2 to about 60. A problem with this approach, however, has been the lack of commercially available standards for the purpose of determining response factors. Since response factors obtained with pulsed amperometric detection for carbohydrates with different degrees of polymerization are known to be very variable (Ammeraal *et al.* 1991), it is not possible to extrapolate response factors from known species to those of unknown chain length. As part of the ENDO project, M Quigley and H Englyst (unpublished results) have overcome this problem by development of a technique for first separating the NDO by high-performance anion exchange chromatography and then using a novel post-column acid hydrolysis technique to convert the NDO into their constituent monosaccharides, thereby allowing the use of readily available monosaccharide standards for quantification.

The non-digestibility of the NDO can be demonstrated *in vitro* by subjecting them to treatment with pancreatic and small-intestinal enzymes, and *in vivo* for human subjects with ileostomized volunteers. These are patients whose large bowel has been removed and who have a stoma at the end of the ileum. In such subjects, digestion is limited to the small intestine, and the remainder of the food bolus is available for collection into a pouch; this is considered to be representative of material that would enter the colon. Carbohydrates are termed indigestible when at least 90 % of the quantity ingested is recovered in the pouch (Knudsen & Hessov, 1995; Ellegård *et al.* 1996).

Very few NDO have been tested for digestibility *in vivo*, but for the inulin-type fructans the data from *in vitro* studies match those obtained *in vivo*. Table 1 shows the chemical structure, molecular size and sources of the NDO studied in this project. Analysis of these preparations indicates that whilst some are very pure, containing 86–87 % NDO e.g. inulin and oligofructose, for others the NDO content is a minor fraction, about 20–30 %, the rest comprising free mono- and disaccharides, starch and even NSP. For example, the xylo-oligosaccharides studied contained (g/kg): 294 NDO, 410 starch and 150 monosaccharides. This is an important factor to bear in mind when interpreting human or animal feeding studies.

Consensus. NDO can be distinguished from other carbohydrates on the basis of their solubility *in vitro* under specified conditions and their resistance to human intestinal enzymes. Methods for the quantitative analysis of NDO in general, and the inulin-type fructans in particular, are available.

Effect on the colonic microflora

NDO escape digestion in the human small bowel and are potential substrates for hydrolysis and fermentation by intestinal bacteria. Three major consequences of colonic fermentation have been studied: (1) the increase in bacterial biomass that leads to an increase in faecal output; (2) the production of short-chain fatty acids as some of the end-products of the fermentation process; (3) the prebiotic effect.

Increase in biomass and stool bulking

State of the art. NDO remain in solution in the chyme and contribute to the osmotic pressure, resulting in increased water flow. NDO are fermented by the colonic microflora, and this results in production of gases, short-chain fatty acids and lactate, which are known to affect gastrointestinal motility (Cherbut *et al.* 1994). The fermentation of NDO results in the production of biomass, theoretically at least 300 g/kg on a dry weight basis (Roberfroid *et al.* 1993), which increases faecal bulk and dry matter.

In a study where eight volunteers were given 15 g oligofructose/d, Gibson *et al.* (1995) observed an increased stool weight corresponding to 1·5–2 g faecal weight increase per gram oligofructose ingested. Intestinal transit time was not affected. E Den Hond, B Geypens and Y Ghoos (unpublished results) confirmed the impact of NDO on bowel habit with long-chain inulin in slightly constipated volunteers. They demonstrated a significant increase in stool frequency, and hence a relief of constipation, in this group of volunteers. They confirmed the 1·5–2 g stool weight increase per gram NDO consumed. Together, these effects result in stimulation of regular bowel habit. In terms of public health, this translates into relief of constipation, which is particularly frequent in the elderly and in young children.

Consensus. There is consistent evidence of an effect of NDO on bowel habit, which results in a typical faecal bulking effect of 1·5–2 g increase per gram NDO ingested, and in a normalization of stool frequency (aiming at daily defecation).

Production of short-chain fatty acids

State of the art. The anaerobic microflora in the colon convert carbohydrates to short-chain fatty acids. As different carbohydrates appear to stimulate growth and metabolic activity of different populations of the colonic ecosystem to different extents, their fermentation results in the formation of different proportions of acetate, propionate and butyrate. *In vitro* fermentations with human faecal or rat caecal microflora indicate that inulin-type fructans typically increase the production of acetate and of butyrate (indicating that populations other than the bifidobacteria also benefit, as bifidobacteria do not produce butyrate), whereas galacto-oligosaccharides increase the production of acetate and propionate, and xylo-oligosaccharides that of acetate only (Campbell *et al.* 1997; Djouzi & Andrieux, 1997).

Prebiotic effect

State of the art. At the start of the ENDO project, the concept of a prebiotic was introduced into nutritional

science (Gibson & Roberfroid, 1995). A prebiotic was defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health.

The only prebiotic effect that has been fully demonstrated is the selective stimulation of the growth of bifidobacteria in complex microbial communities that exist in the colon. Bifidobacteria are generally classified as health-promoting and, along with lactobacilli, are used as probiotics. No harmful effect related to their oral intake has been reported, but work is required to demonstrate specific health effects. Hitherto, the only NDO for which such a demonstration has been made convincingly, both *in vitro*, and most importantly in human subjects *in vivo,* appear to be the inulin-type fructans inulin and oligofructose (Roberfroid *et al.* 1998; see Table 3). These prebiotic ingredients are regarded as potentially beneficial for health because they lead to an increase in barrier function and reduction in risk of intestinal infections.

ENDO contribution. The main contribution of the ENDO project to the prebiotic concept concerned the development of methodological approaches to study such effects. The prebiotic effect of NDO has been studied by means of both *in vitro* and human *in vivo* experiments.

Prebiotic effect: in vitro *experiments*

These experiments concern fermentations carried out in small bioreactors inoculated with pure bacterial strains, reconstituted mixtures or faecal slurries. Variables such as temperature, pH and nutrient composition are carefully controlled. This technique allows study of the influence of NDO on the bacterial composition of mixed bacterial faecal slurries as well as the fermentation of NDO by pure bacterial cultures.

The different compartments of the colon can be simulated with continuous culture experiments with (sequences of) chemostats (Macfarlane *et al.* 1998). One limitation of this type of experiment is that metabolites are not withdrawn from the environment as they are in the colon. The accumulation of these products affects the equilibrium of many ongoing biochemical processes (inhibition). It is not possible to quantify the influence of the artificial environment of a bioreactor. Nevertheless, the relative comparisons that can be studied are considered important tools in prebiotic research, as they allow the demonstration (qualitatively) of certain mechanisms, leading to the formulation of hypotheses that can be tested by well-designed human feeding studies.

In the ENDO project, an *in vitro* gnotoxenic (bacterial flora with known composition) fermentation chemostat proved to be a suitable model for studying bacterial interactions (barrier effect or colonization resistance; D Newton, J Cummings,

Table 2. Overview of the evolution of *in vitro* fermentation experiments with non-digestible oligosaccharides (NDO), leading to the assumption that NDO are prebiotic compounds

Reference	Model	Substrate	Effect
Wada (1990)	Test-tube; pure cultures (various)	Inulin, oligofructose, glucose	pH measurements gave a first indication of the selective promotion of Bifidobacteria
Wang & Gibson (1993)	Batch; faecal slurry as inoculum	Glucose, galactose, arabinose, fructose, lactose, sucrose, cellobiose, lactulose, oligofructose, inulin, starch, pectin, arabinogalactan, sorbitol, lactitol, maltitol, polydextrose	Pronounced selective increase in Bifidobacteria demonstrated Monitoring of SCFA production revealed high acetate (and lactate) production with oligofructose
Gibson & Wang (1994*a*)	Batch; pure culture (Bifidobacteria, *E. coli*, *C. perfringens*, Bacteroides)	Inulin, oligofructose, branched oligofructose, glucose	Growth rate of Bifidobacteria on oligofructose>inulin>branched oligofructose>glucose. In the non-Bifidobacteria pure cultures, fastest growth was observed on glucose
Gibson &Wang (1994*b*)	Batch; diffusion chemostat. Single chemostat, inoculated with pure cultures	Oligofructose	Demonstration of inhibitory effects of Bifidobacteria on the growth of other colonic bacteria, possibly explaining enhanced bifidogenic effect
McBain & MacFarlane (1997)	Three-stage chemostat; methanogen-free faecal slurry as inoculum	Inulin	Selective increase of Bifidobacteria Decrease of Clostridia
Hopkins *et al.* (1998)	Batch; pure culture with *Bifidobacterium* spp.	Xylo-OS, galacto-OS, soyabean OS, inulin, pyrodextrin, oligofructose	Measurement of maximal specific growth rate (μmax) revealed significant interspecies differences depending on growth substrates
Newton *et al.**	Chemostat; defined population of fifteen pure cultures representing the most abundant intestinal bacteria	A mixture of inulin, pectin, xylan, arabinogalactan and starch	Validation of the model allowing study of bacterial interactions (e.g. colonization resistance)

OS, oligosaccharides; SCFA, short-chain fatty acids.
* D Newton, J Cummings, S Macfarlane and G Macfarlane, unpublished results.

S Macfarlane and G Macfarlane unpublished results). Table 2 summarizes the *in vitro* microflora experiments.

Consensus. The results obtained from *in vitro* fermentation experiments (batch and continuous cultures inoculated either with faecal slurries or pure cultures) are important in prebiotic investigations, particularly when integrated with information from human *in vivo* studies. Combination of these data provides valuable insight into the mechanisms of prebiotic action. *In vitro* models alone cannot be considered adequate for study of the complex ecosystem of the colon.

Pure culture results cannot be used to show a prebiotic effect. Indeed, in such conditions most NDO are fermented by miscellaneous species and strains, but these findings do not preclude selective stimulation among complex mixtures of micro-organisms. A simplified chemostat model of the colonic microbiota (with a known population of the fifteen most abundant intestinal bacteria) shows exceptional promise for elucidating the underlying principles associated with bacterial cell–cell interactions (barrier effect or colonization resistance).

As a result of the discussion of the available scientific data, the authors propose the following definition: 'A prebiotic effect is a food-induced increase in numbers and/or activity predominantly of bifidobacteria and lactic acid bacteria in the human intestine'. This definition is an adapted version of that of Gibson & Roberfroid (1995) and implies that besides bifidobacteria and lactic acid bacteria, some ('selectivity') other bacteria may be stimulated. However, the definition incorporates the bifidobacteria and the lactic acid bacteria because they are considered good biomarkers of a well-balanced intestinal flora. The health aspect is omitted from the definition, because to date no information is available which could support such a statement.

Prebiotic effect: in vivo *human nutrition studies*

State of the art. Table 3 lists published and scientifically sound (criteria are faecal sample pretreatment, confirmation of the identity of counted colonies, control of dietary intake) studies investigating the bifidogenic properties of various types of NDO.

Table 3. Overview of human nutrition studies investigating the prebiotic (bifidogenic) properties of different non-digestible oligosaccharides (NDO)

Reference	Dose (g/d) Type of NDO	No. volunteers Age (years) Supplement period	Control of bacteriological measurement	Remarks on protocol	Log increase in Bifidobacteria	Statistical significance	Indication of selectivity
Bouhnik et al. (1996a)	12·5 Oligofructose	20 (10M/10F) 22–39 12 d	Selectivity of medium	Samples at 4° for <12 h	1·2	P<0·01	Only Bifidobacteria and total anaerobes measured
Bouhnik et al. (1997)	10·0 TOS	8 (4M/4F) 24–32 21 d	Selectivity of medium	–	1·1	P<0·05	Bifidobacteria increased whereas enterobacteria were not affected
Gibson et al. (1995)	15 Oligofructose	8 (M) 20–25 2 weeks	Microscopy and fermentation	Fresh faecal samples	0·7	P<0·01*	Decrease of bacteroides and *Clostridium* No change in other groups
Gibson et al. (1995)	15 Inulin	4 (M) 20 2 weeks	Microscopy and fermentation	Fresh faecal samples	0·9	P<0·001	No change in other groups
Ito et al. (1993)	15 TOS	12 (M) 27–43 1 week	Microscopy	Fresh faecal samples	0·25 (high initial counts)	P<0·01 (increase in lactobacillus P<0·05)	Bacteroides and clostridia decreased
Kleessen et al. (1997)	20 and 40 Inulin	10 (F) 68–89 19 d	Selectivity of medium	Stored faecal samples at –20°	0·90 and 1·30	P<0·05	Decreased bacteroides; no change in other groups
Menne & Guggenbuhl (1997)	8 F$_n$-type oligofructose	8 (5M/3F) 20–50 2 weeks	Microscopy	Fresh faecal samples	0·9	P<0·05*	No change in other groups
Mitsuoka et al. (1987)	8 Oligofructose	23 (M) 50–90 2 weeks	Colony type microscopy	–	0·9	P<0·005*	Decrease in enterobacteria; other measured groups remained constant
Rochat et al.†	8 Oligofructose	38 (M) Adult 2 weeks	Selectivity of medium	Fresh faecal samples	1·35	P<0·01 (increase in lactobacillus P<0·05)	Decrease of clostridia Others not affected
Buddington et al. (1996)	4 Oligofructose	12 (6M/6F) 20–34 25 d	Selective media/ colony type	Fresh faecal samples	0·8	P<0·03*	No distinct evidence
Alles et al. (1998b)	7·5 and 15 TOS	40 (22M/18F) 25–55 3 weeks	Selectivity of medium	Fresh faecal samples (<3 h)	No significant increase	–	Gut flora remained unaffected

M, Male; F, Female; TOS, transgalactosylated oligosaccharides.
* Higher counts of lactic acid bacteria observed on adminstration of NDO.
† F Rochat, N Medjoubi, G Rumo and C Heer, unpublished results.

Considering this plethora of data on the bifidogenicity of inulin and oligofructose, the authors reached consensus on the conclusion that inulin-type fructans or $\beta(2 \rightarrow 1)$ fructans, independent of chain length, all have significant prebiotic properties. There is no difference in prebiosis between oligofructose obtained by synthesis from sucrose and that obtained by partial enzymic hydrolysis from chicory inulin (Gibson & Wang, 1994*b*; Bouhnik *et al.* 1996*a*; Buddington *et al.* 1996).

Partial enzymic hydrolysis of inulin (molecules composed of $\beta(2 \rightarrow 1)$-linked fructose chains with lengths varying between three and sixty monomers attached to a single glucose unit) results in the formation of GF_n and F_n-type fructans (where G is glucose, F is fructose and n is the number of fructose moieties). Menne & Guggenbuhl (1997) observed a bifidogenic effect with F_n-type fructans that was similar to earlier observations with GF_n-type fructans. Roberfroid *et al.* (1998) concluded from their compilation work that in a large population there seems to be no dose–response effect of these NDO on the increase in numbers of bifidobacteria, for daily intake doses between 4 and 40 g/d. This is because the bifidogenic effect is inversely related to the volunteer's initial bifidus count.

In all of these carefully executed experiments, a significant bifidogenic, and hence prebiotic, effect of inulin and/or oligofructose has been observed. Three human studies have been performed with transgalactosylated oligosaccharides (Ito *et al.* 1993; Bouhnik *et al.* 1997; Alles *et al.* 1998*b*), of which two demonstrated a prebiotic effect and one showed no effect.

The consistently observed prebiotic potential of inulin-type fructans may be ascribed to their molecular structure: linear chains composed of mainly $\beta(2 \rightarrow 1)$-linked fructose molecules. The other types of NDO are either branched, and yet less readily accessible for bacterial hydrolysis, or composed of several types of glycosidic bonds, which makes induction of specific enzyme systems to degrade them less efficient.

Consensus. There is now strong evidence, based on observations with over 100 volunteers differing in sex, age, race and dietary habits, showing that $\beta(2 \rightarrow 1)$-type fructans are prebiotic (Roberfroid *et al.* 1998). Few human feeding studies investigating the bifidogenic potential of galacto-oligosaccharides seem to indicate that they may have a prebiotic potential. There are preliminary animal studies indicating prebiotic effects of soyabean oligosaccharides. A significant fraction of these compounds is absorbed in the small intestine, and thus becomes unavailable for colonic fermentation. Very little scientific information on xylo-oligosaccharides and pyrodextrins is available.

Perspectives. To perform human prebiosis studies is extremely laborious and time-consuming. In these studies, the classical plate-counting techniques are the bottle-neck. Studies designed to develop and validate quantitative techniques making use of 16 S rRNA probes to assess the microflora are underway.

Another technique that might prove to be useful is the MIDI system (an acronym of MIcrobial IDentification; a Corporation in Barksdale Professional Center, Newark, DE, USA), which uses cellular fatty acids as bacterial biomarkers. The system is GC-based, and is interfaced to a computer containing libraries for several hundred different bacterial species. The organisms, in pure or mixed cultures, are identified on the basis of their whole-cell fatty acid (as methyl ester) profiles. Reproducible markers have been identified for particular groups of bacteria (e.g. Bacteroides, bifidobacteria, clostridia; D Newton, J Cummings, S Macfarlane and G Macfarlane, unpublished results). These less laborious techniques are promising tools for executing human prebiosis studies on a larger scale (greater number of volunteers, and longer study periods).

Effect of non-digestible oligosaccharides on mineral absorption

State of the art. There is increasing evidence that NDO improve the bioavailability of minerals. Ohta *et al.* (1993, 1994, 1995), Delzenne *et al.* (1995), Scholz-Ahrens *et al.* (1998) and Lemort & Roberfroid (1997) published the results of rat studies in which increased Ca and Mg absorption following consumption of inulin-type fructans was observed. Baba *et al.* (1996) formulated the hypothesis that the effects of NDO on Ca and Mg absorption occur at the level of the large intestine. This represents a new concept, as it is generally accepted that mineral absorption occurs mainly via the small intestine. Using ovariectomized rats functioning as a model for postmenopausal women, Scholz-Ahrens *et al.* (1998) observed a dose effect of inulin on increased Ca absorption and demonstrated that the increased amount of absorbed Ca was effectively used to increase bone mineral density. This opens the way to considering consumption of inulin to reduce the risk of osteoporosis.

Further, it was observed by Delzenne *et al.* (1995) in normal rats and by Ohta *et al.* (1995) in anaemic rats that intake of inulin-type fructans increased the blood Fe content. Chonan & Watanaki (1995) reported an increase in Ca^{2+} absorption in rats that were administered transgalactosylated oligosaccharides. Ellegård *et al.* (1996), in studies with ileostomized volunteers who were administered inulin and oligofructose, observed that the intake of NDO did not alter the transition of Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} from the ileum into the colon. This suggests that the altered mineral absorption is due to events taking place in the colon.

ENDO contribution. Two human feeding studies were performed during the ENDO project. van Dokkum *et al.* (1995) optimized a stable-isotope technique, which was applied to healthy young male adults consuming 15 g inulin, oligofructose or galacto-oligosaccharides/d. The study did not demonstrate an effect of NDO on mineral absorption. When reinvestigating the protocol, it was thought that the sampling period for the inulin studies (24 h) may have been too short to take into account colonic effects. Noting that all previous rat experiments were carried out with growing animals, a second study was performed with a group of male adolescents. It is thought that the mineral absorption rate is highest during adolescence and that it determines acquired bone mineral density. The colonic part of Ca^{2+} absorption was included by extending the collection of urine to 36 h after isotope administration.

Twelve volunteers consumed 15 g oligofructose/d for 1 week. A significant increase ($+25\%$; $P < 0.05$) in Ca^{2+}

Table 4. Overview of human feeding studies investigating the effect on mineral absorption of non-digestible oligosaccharides (NDO) consumption

Reference	NDO Dose (g/d) Intake period (weeks)	No. of male volunteers Age (years)	Method of measurement	Result	Statistical significance
Coudray et al. (1997)	Inulin 40 4	9 19–24	Apparent mineral absorption	Ca, 58 % increase (from 21·3 to 33·7 %) Mg, Fe, Zn, no effect	$P < 0.01$
van den Heuvel et al.*	Oligofructose, inulin, TOS 15 3	12 20–26	True mineral absorption (24 h urine collection; may have been too short)	No altered Ca or Fe absorption with any NDO tested	–
van den Heuvel et al. (1997) and *	Oligofructose 15 1	12 15–16	True mineral absorption monitored with stable isotopes (this time with longer urine sampling period of 36 h)	Ca, 26 % increase (from 47·8 to 60·1 %)	$P < 0.05$

TOS, transgalactosylated oligosaccharides.
* EGH van den Heuvel, Th Muys, W van Dokkum and G Schaafsma, unpublished results.

absorption was observed as compared with a placebo (van den Heuvel *et al.* 1999).

During the execution of the ENDO project, Coudray *et al.* (1997) observed an increase (+58 %; $P < 0.01$) in apparent Ca absorption in a group of nine adult volunteers who consumed 40 g inulin/d (Table 4).

Consensus. The stimulation of mineral (Ca^{2+}, Mg^{2+} and Fe^{2+}) absorption by inulin-type fructans and transgalactosylated oligosaccharides has been repeatedly confirmed in rat studies. The use of different models indicates that the increased absorption originates mainly at the level of the large intestine and effectively results in increased bone mineral density. There is promising evidence that the consumption of inulin-type fructans results in increased Ca^{2+} absorption in human subjects. Two human feeding studies showed a significant effect, whereas in another study no effect was observed, but this may have been due to a suboptimal study design. Results from animal models indicate similar effects for Mg^{2+} and Fe^{2+} absorption. There is limited evidence for effects of transgalactosylated oligosaccharides in animal models only. There are no data available indicating comparable effects for other NDO.

Effect of non-digestible oligosaccharides on lipid metabolism

At the start of the ENDO project it was known that feeding rats on a diet supplemented with inulin-type fructans induces a hypolipidaemic and, more particularly, a hypotriacylglycerolaemic effect (Fiordaliso *et al.* 1995). It was one of the objectives of the ENDO project to study this effect in more detail and potentially to confirm it in human volunteers.

State of the art. Lowering of serum triacylglycerols in rats has been reported by Hata *et al.* (1983) and Delzenne *et al.* (1993) with inulin-type oligosaccharides (100 g/kg dose level) and by Kaneko *et al.* (1992) with isomaltooligosaccharides. Cholesterol levels were not always reduced; when they were, this was observed only after a prolonged feeding period (>12 weeks).

Yamashita *et al.* (1984) described a reduction of LDL-cholesterol and fasting blood glucose levels in eighteeen non-insulin-dependent diabetic subjects given oligofructose (8 g/d for 14 d). They observed that the reduction was

greater in hypercholesterolaemic subjects. No effect on circulating triacylglycerols was seen.

Alles *et al.* (1998a) repeated the experiment with 15 g oligofructose/d for 20 d. They observed no effect on fasting circulating lipid variables, nor on fasting glucose levels. A postprandial study was not performed in any of these studies.

A study with healthy but slightly hyperlipidaemic subjects by Davidson *et al.* (1998) resulted in a regulating effect of inulin (18 g/d) on total and LDL-cholesterol levels. However, the experimental protocol was biased by the study food used, which did not allow the observations to be attributed unequivocally to the inulin intake.

Canzi *et al.* (1995) observed that the intake of inulin (9 g/d) resulted in a reduction in serum triacylglycerol and cholesterol levels, whereas Pedersen *et al.* (1997) observed no effect with inulin (14 g/d) in a group of healthy normolipidaemic women. Recently, Williams (1998) performed a human feeding study with fifty-four healthy but slightly hyperlipidaemic volunteers. They were administered 10 g long-chain inulin/d. There were no effects on cholesterol, but fasting serum triacylglycerols (−19 % after 8 weeks) and insulin levels (−17 % after 4 weeks and −10 % after 8 weeks) dropped significantly.

ENDO contribution. Studies with isolated hepatocytes showed reduced hepatic lipogenic activity in NDO-fed rats, and led to the observation that the activity of all hepatic lipogenic enzymes was significantly downregulated (−50 %) in the $\beta(2 \rightarrow 1)$ fructan-fed rats (Kok *et al.* 1996b). This may be due to modified genetic regulation (gene expression) in the liver. Further research revealed that this might be induced by hormonal changes (insulin, glucagon-like protein of type 1 and glucose-dependent insulinotropic polypeptide; Kok *et al.* 1998a). It is hypothesized that bacterial metabolites (more particularly propionate) from NDO fermentation are involved (Alamowitch *et al.* 1993). Further, it was demonstrated by Kok *et al.* (1998b) that the increase in postprandial triacyglycerol levels that is induced by a fat-rich (Western-type) diet is decreased (−50 %) in rats on oligofructose feeding, and that the increase in liver triacylglycerol induced by a high fructose load is suppressed in oligofructose-fed rats (Kok *et al.* 1996a).

Controlled feeding studies by van Dokkum *et al.* (1995) with normolipidaemic healthy volunteers resulted in no effect with 15 g oligofructose, inulin or transgalactosylated oligosaccharides/d during a period of 3 weeks. The blood lipid variables of these subjects were optimal, and according to recent findings by Williams (1998), the feeding period rather short. It is worthwhile noting that the intake of NDO did not negatively affect the blood lipid variables.

In a second ENDO study by van Vliet (1997) with a group of fifty slightly hyperlipidaemic male volunteers, of whom twenty-five received 15 g oligofructose/d for 3 weeks, and twenty-five were given control food, no effect on fasting serum lipid variables was observed. From these data, it appears that to elucidate any effect of NDO on the many mechanisms (bile acid metabolism, intestinal flora, liver lipid metabolism, hormone status, which are linked to nutrition status, health condition, age, sex, etc.) that interact with lipid metabolism, the study design is of utmost importance. The studies described in Table 5 cannot be compared directly, as they all differ in some aspects.

Consensus. There is preliminary evidence from animal studies that lipid metabolism is affected by inulin-type fructans. The experimental design for human nutrition studies is critical if effects are to be demonstrated unambiguously. The data available at present are still inconsistent, but indicate that the intake of moderate levels of inulin or oligofructose may affect human lipid metabolism. They are the basis for further ongoing studies investigating other aspects of lipid metabolism (insulin and incretins, obesity, diabetes). There is no indication of an effect on lipid metabolism in human subjects for transgalacto-oligosaccharides or any other type of NDO.

Interaction of non-digestible oligosaccharides with the carcinogenesis process and immunology

This topic was not part of the ENDO project but available experimental studies were discussed by the authors during the consensus report meeting.

State of the art. Only evidence from animal experiments is available. Reddy *et al.* (1997) added 100 g long-chain inulin and oligofructose/kg to the diet of rats. After adaptation, the animals were injected with azoxymethane. For both types of fructan, a statistically significant reduction in the numbers of colonic aberrant crypt foci was observed. These preneoplastic lesions are early indicators of future risk of tumour development (Table 6).

Rowland *et al.* (1998) administered 50 g long-chain inulin/kg diet to rats following azoxymethane injection

Table 5. Summary of published human nutrition studies (including ENDO project results) of the effect of non-digestible oligosaccharides (NDO) on lipid metabolism

Reference	NDO Dose (g/d) Intake period (d)	Number of volunteers Type Age (years)	Study design	Fasting serum levels	Postprandial measurements
Yamashita *et al.* (1984)	Oligofructose 8 14	28 (13M/15F) NIDD 47 (SD 7)	Parallel (18 subjects on study food; 10 on placebo) Patients not on medication	↓Total-C ↓LDL-C =HDL-C =TG ↓Glucose	NM
Alles *et al.* (1998a)	Oligofructose 15 20	20 (9M/11F postmenopausal) NIDD ?	Cross-over; no washout Patients on blood glucose-lowering medication	No effect	NM
Canzi *et al.* (1995)	Inulin 9 28	12 M Normolipidaemic 23 (SD 0·5)	Run-in (placebo) of 4 weeks; 4 weeks inulin-containing study food	↓Total-C ↓LDL-C =HDL-C ↓TG NM Glucose	NM
Pedersen *et al.* (1997)	Inulin 14 28	64 F Normolipidaemic 'Young'	Cross-over study without washout period	Reduced LDL:HDL ratio; no effect on Total-C, TG or glucose	NM
van Dokkum *et al.* (1995)	Inulin, oligofructose, TOS 15 21	12 M Normolipidaemic 23 (SD 3)	Latin square; no washout Controlled diet	No effect	Glucosaemia and insulinaemia: no difference with placebo detected
Davidson *et al.* (1998)	Inulin 18 42	24 (12M/13F, Caucasian) Hyperlipidaemic 60 (SD 5)	Double-blind cross-over with 6-week washout period Volunteers on low-fat diet (fat < 30 % of energy)	↓Total-C ↓LDL-C =HDL-C =TG NM Glucose	NM
van Vliet (1997)	Oligofructose 15 21	50 M Hyperlipidaemic (TG 2·5 (SD 0·9) mmol/l) 50 (SD 7)	Parallel (25 subjects on study food and 25 on placebo) No controlled diet	No effect	Glucosaemia and insulinaemia: no difference with placebo detected
Williams (1998)	Long-chain inulin 10 56	54 (M/F) Hyperlipidaemic (TG 1·2–3 mmol/l, TC 5·5–8 mmol/l) 35–70	Double-blind randomized placebo-controlled	=Total-C =LDL-C =HDL-C ↓ TG	=Glucosaemia ↓ fasting insulin levels during inulin intake

C, cholesterol; Total-C, LDL-C+HDL-C; TG, triacylglycerols; TC, total cholesterol; NM, not monitored; M, Male; F, Female; ↓, reduction; =, no change; NIDD, non-insulin-dependent diabetic.

Table 6. Overview of animal studies demonstrating the chemopreventive action of non-digestible oligosaccharides (NDO) in colon cancer

Reference	Type of NDO Dose	Animal Numbers	Protocol	Observation
Gallaher *et al.* (1996)	OF (20 g/kg diet) *Bifidobacterium* spp. (10^8) OF+bifidobacteria	Rat 15/group	DMH injections before administering study food	Decrease in ACF with OF + Bif > OF > Bif (synbiotic effect)
Reddy *et al.* (1997)	Long-chain inulin OF 100 g/kg diet	Rat 12/group	NDO feeding started 2 weeks before AOM injections. Counting of ACF 8 weeks after last AOM treatment (impact on initiation and on propagation phase)	Inulin, −35 % ACF** OF, −24 % ACF*
Taper *et al.* (1997)	OF, inulin, pectin 150 g/kg diet	Mouse 9–12/group	Tumour cell lines (EMT6 and TLT) implanted in the thigh	Mean tumour surface increase as a measure for growth was decreased
Pierre *et al.* (1997)	OF 58 g/kg diet	Min mouse 5/group	Min mice spontaneously develop intestinal tumors (mainly in the small intestine)	Fewer tumours in the small intestine (**) and in the colon (**); stimulation of GALT (*)
Rowland *et al.* (1998)	Long-chain (LC) inulin (50 g/kg diet) *Bifidobacterium longum* (10^8) LC inulin+*B. longum* placebo	Rat 15/group	AOM injections before administering study food (impact on propagation phase)	Inulin, −21 % ACF* *B. longum,*−29 % ACF* Inulin + *B. longum*, −74 % ACF*** (synbiotic effect)

ACF, total colonic aberrant crypt foci; AOM, azoxymethane; DMH, 1,2-dimethylhydrazine; GALT, gut-associated lymphoid tissue; OF, oligofructose; Bif, bifidobacteria.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(post-initiation experiment). They observed a significant reduction in the numbers of colonic aberrant crypt foci, and an inhibition by inulin of the propagation phase of the carcinogen. A comparable effect was induced on administration of bifidobacteria. When long-chain inulin and bifidobacteria were administered simultaneously, a synergistic reduction of the numbers of aberrant crypt foci was obtained. Gallaher *et al.* (1996) observed a comparable synbiotic effect with oligofructose and bifidobacteria.

Taper *et al.* (1997) fed a diet containing 150 g oligofructose or inulin/kg to mice bearing intramuscularly implanted tumour cells from two lines (liver tumour cells, TLT; and a mammary carcinoma cell line, MT6) and demonstrated that growth of these tumours was significantly reduced compared with placebo. The same tumour cell lines injected into the peritoneum of mice develop into ascitic tumours. The mean survival time of ascitic tumour-bearing mice which were fed with either inulin or oligofructose was significantly increased compared with placebo-fed mice (Taper *et al.* 1998).

Pierre *et al.* (1997) observed a significant reduction in the numbers of spontaneously developing tumours (mainly in the small intestine) in Min-mice associated with oral intake

of oligofructose (58 g/kg diet). They reported that the intestinal immune system of the mice was stimulated.

Consensus. The available data, based on research with inulin-type fructans and originating from various types of animal models, consistently demonstrate a reduced risk in experimentally induced carcinogenesis processes. It was thought that these data are sufficient to claim preliminary evidence for interaction of NDO with colon carcinogenesis in experimental animals. The data therefore support the investment for performing human nutrition studies.

Conclusion

The present compilation of research data (Table 7) emphasizes that NDO, which are present in a Western-type diet in only small quantities, have potential for improving health.

At present there is convincing evidence that: (1) NDO positively affect the composition and metabolic activity of the intestinal microflora (prebiosis); (2) NDO consumption stimulates bowel habit; (3) a daily moderate supplement of NDO stimulates mineral (especially Ca and Mg) absorption, and thus has the potential to prevent or postpone osteoporosis.

Table 7. Consensus on the different functional food effects of non-digestible oligosaccharides in decreasing order of established evidence in human studies

Effect	Evidence (in human subjects)
Prebiotic and interaction with intestinal flora	Strong
Regulation of bowel habit, stool bulking, and increase of stool frequency	Strong
Increased mineral absorption	Promising
Impact on lipid metabolism	Preliminary, data still inconsistent
Colon cancer	No human data available (experimental animals, preliminary)

Insufficient evidence is available from human studies to state that NDO systematically improve blood lipid variables. However, ongoing studies may produce more definitive statements.

Promising observations in animal models concerning chemoprevention and retardation of tumour growth in peripheral tissues have been published. This topic deserves more in-depth research and human studies are required.

Acknowledgements

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